The Amendments

New claims 45-47 have been added to specify embodiments comprising certain antibody fragments as the ligand. The claims introduce no new matter. In claim 45 the ligand is a fragment of an antibody comprising a portion of an immunoglobulin light chain variable region and a portion of an immunoglobulin heavy chain variable region. In claim 46, the fragment comprises a light chain variable region and a heavy chain variable region. Support for claims 45 and 46 is found in the specification at page 6, lines 14-16 ("The fragment can comprise, for example, at least a portion of an immunoglobulin light chain variable region and at least a portion of an immunoglobulin heavy chain variable region"). In claim 47 the ligand comprises a single chain Fv fragment of an antibody. Support is found at page 6, lines 17-20 ("the ligand can be a synthetic polypeptide comprising at least a portion of an immunoglobulin light chain variable region and at least a portion of an immunoglobulin heavy chain variable region) and page 13, lines 6-7 ("Single chain Fv (scFv) constructs based on the L8A4 variable region can also be used instead of the whole L8A4 antibody").

The Rejection of Claims 1-4, 8, 11-21, and 44 Under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 8, 11-21, and 44 stand rejected. The office action states that the claims are enabled for ligands consisting of antibodies but not for fragments of antibodies or synthetic peptides. According to the office action, the specification does not teach a labeled oligopeptide which is covalently bound to a small antibody fragment or a synthetic polypeptide. This rejection is respectfully traversed.

The office action asserts that undue experimentation would be required to design antibody fragments or synthetic polypeptides which retain an antigen-binding conformation

when used in the context of the invention. Two specific concerns are expressed in the office action: (1) it is allegedly unclear whether the required three-dimensional structure for binding a cell surface antigen will be disrupted in producing a given fragment or polypeptide; and (2) it is allegedly unpredictable whether the attachment of an oligopeptide will disrupt the structure of the ligand and adversely affect antigen binding.

Applicant respectfully maintains that the subject claims are enabled because one of ordinary skill could readily make and use the antibody fragment or synthetic polypeptide of the subject claims based on the specification and the state of the art without undue experimentation. The specification at page 6, lines 12-17, contemplates any fragment of an antibody or any synthetic polypeptide which specifically binds a cell surface antigen:

> If a fragment of an antibody is used, the fragment should be capable of specific binding to a cell surface antigen. The fragment can comprise, for example, at least a portion of an immunoglobulin light chain variable region and at least a portion of an immunoglobulin heavy chain variable region. A ligand can also be a synthetic polypeptide which specifically binds to a cell surface antigen.

Well-defined fragments of antibodies which retain specific antigen binding are common in the art. The passage cited above describes antibody fragments that comprise portions of both light and heavy chain variable regions. It is well understood that antigen binding is specified within the light and heavy chain variable regions and that any fragment which preserves those regions will very likely possess antigen binding characteristics similar to the intact antibody from which they were derived. Two common fragments with the required properties are the Fab and $F(ab')_2$ fragments, produced by proteolytic cleavage using papain and pepsin, respectively. *See* Charles A. Janeway, *Immunobiology. The Immune System in Health and Disease*, 4th Ed. (1999), at pages 82-83 (Section 3-3, entitled "The antibody molecule can readily be cleaved into functionally distinct fragments", and Fig. 3.4; copy enclosed). The production of Fab and $F(ab')_2$

fragments is routinely practiced in the art using limited proteolysis conditions which generally permit retention of specific antigen-binding activity. This is possible because the proteases first attack at the exposed hinge region, while the antigen binding site is located in the more rigid and compact variable regions which remain protected against limited proteolysis.

The office action overstates the problem of predicting the conformation of antigen-binding domains of antibody fragments. In fact, the field has advanced to a substantial degree of predictability, based largely on the fact that only the hypervariable segments assume unpredictable conformations, while the bulk of the so-called "variable region" of antibodies actually forms a compact, well-conserved structure. For example, one U.S. patent describes the art as acknowledging the stable three dimensional structure of the variable region:

> [I]t is by now well established that all antibodies of a certain class and their Fab fragments whose structures have been determined by X-ray crystallography, even when from different species, show closely similar variable regions despite large differences in the hypervariable segments. The immunoglobulin variable region seems to be tolerant toward mutations in the combining loops. Thereafter, other than in the hypervariable regions, most of the so-called "variable" regions of antibodies, which are defined by both heavy and light chains, are in fact quite constant in their three dimensional arrangement. See, for example, Huber, R. (Science 233:702-703 (1986)).

U.S. Patent 5,260,203 at column 2, lines 23-34. Furthermore, recombinant techniques have made it possible to reduce the antigen-binding portion of an antibody to a minimal structure, known as "single chain Fv", which comprises parts of the variable regions of both light and heavy chains linked by a synthetic peptide. Description of these fragments can be found in commonly-available immunology textbooks. For example:

> Genetic engineering techniques also now permit the construction of a truncated Fab comprising only the V region of a heavy chain linked by a stretch of synthetic peptide to a V region of a light chain. This is called **single-chain Fv**, named from **F**ragment **v**ariable. Fv molecules may become valuable therapeutic agents because of their small size, allowing ready tissue

> penetration. They may be coupled to protein toxins to yield immunoglobulins with potential application, for example, in tumor therapy.

Janeway, *supra*, at page 83; emphasis original. Indeed, single chain Fv structures are contemplated as part of the instant invention and are described in the specification *inter alia* at page 6, lines 17-21:

> For example, the ligand can be a synthetic polypeptide comprising at least a portion of an immunoglobulin light chain variable region and at least a portion of an immunoglobulin heavy chain variable region, as described in U.S. Patent 5,260,203 or as otherwise known in the art.

U.S. Patent 5,260,203 describes in detail a method of preparing single chain Fv fragments with conserved three-dimensional structure:

> The present invention starts with a computer based system and method for determining and displaying possible chemical structures (linkers) for converting two naturally aggregated but chemically separate heavy and light (H and L) polypeptide chains from the variable region of a given antibody into a single polypeptide chain which will fold into a three-dimensional structure very similar to the original structure made of two polypeptide chains.

U.S. Patent 5,260,203 at column 2, lines 56-63.

In some embodiments, the ligand is a synthetic polypeptide which is unrelated to immunoglobulins. A variety of well-known polypeptide ligands exist (*e.g.*, peptide hormones, inhibitors, drugs, and the like) which bind to cell surface receptors. It is a simple matter for one of ordinary skill to select a polypeptide ligand which is known to specifically bind to a cell surface receptor (cell surface antigen). If amino acid substitutions, deletions, or additions are subsequently made, then the ordinary skilled artisan would know to check whether specific binding activity is affected using a routine binding assay. Furthermore, the portions of polypeptide ligands which are critical for receptor binding are generally known. It is therefore well understood in the art that important amino acid residues in binding portions of a polypeptide

ligand can be chemically blocked during reactions to couple the ligand to an oligopeptide of the invention. There is no need for undue experimentation.

The Office Action states that one of skill in the art would not be able to predict whether non-covalent interactions between the oligopeptide and the ligand would alter the shape of the ligand and thereby interfere with the ability of the ligand to specifically bind the surface antigen. However, any such interactions which disrupt specific binding of the ligand to the surface antigen can be detected using a routine binding assay. In such cases the ligand can be rejected in favor of another.

The production and use of cell-binding ligands which have been labeled through the addition of various chemical moieties is routine, and many are described in the literature. Many such ligands comprise antibody fragments or synthetic peptides. In order to illustrate how routine the use of such ligands is, the following list of publications was selected from Applicant's own bibliography; these articles describe a variety of labeled ligands (identified above each citation) which were routinely prepared and used in research projects.

<u>$F(ab')_2$ and Fab antibody fragments</u>
Colcher, D., Zalutsky, M.R., Kaplan, W., Kufe, D., and Schlom, J.: Radiolocalization of human mammary tumors in athymic mice by a monoclonal antibody. Cancer Res. 1983; 43:736-742.

<u>$F(ab')_2$ antibody fragments</u>
Zalutsky, M.R., and Narula, A.S.: Radiohalogenation of a monoclonal antibody using an N-succinimidyl 3-(tri-n-butylstannyl) benzoate intermediate. Cancer Res. 1988; 48:1446-1450.

<u>$F(ab')_2$ antibody fragments</u>
Colapinto, E.V., Humphrey, P.A., Zalutsky, M.R., Groothuis, D.R., Friedman, H.S., de Tribolet, N., Carrel, S., and Bigner, D.D.: Comparative localization of murine monoclonal antibody Me1-14 F(ab')2 fragment and whole IgG2a in human glioma xenografts. Cancer Res. 1988; 48:5701-5707.

F(ab')$_2$ antibody fragments
Zalutsky, M.R., Garg, P.K., Friedman, H.S., and Bigner, D.D.: Labeling monoclonal antibodies and F(ab')2 fragments with the alpha particle emitting nuclide astatine-211: preservation of immunoreactivity and in vivo localizing capacity. Proc. Natl. Acad. Sci. USA 1989; 86:7149-7153.

Alpha-melanocyte stimulating hormone (a polypeptide)
Garg, P.K., Alston, K.L., Welsh, P.C., and Zalutsky, M.R.: Enhanced binding and inertness to dehalogenation of "-melanotropic peptides labeled using N -succinimidyl 3-iodobenzoate. Bioconjugate Chem., 1996; 7:233-239.

scFv antibody fragments
Kuan, C.T., Reist, C.J., Lorimer, I.A.J., Pegram, C., Pastan, I., Zalutsky, M.R., and Bigner, D.D.: Biodistribution of 125I-labeled anti-EGFRvII single-chain Fv exhibits rapid, specific and high-level targeting of xenografts. Clin. Cancer Res. 1999; 5:1539-1549.

Octreotide (a polypeptide)
Vaidyanathan, G., Affleck, D., Welsh, P., Srinivasan, A., Schmidt, M., and Zalutsky, M.R.: Radioiodination and astatination of octreotide by conjugation labeling. Nucl. Med. Biol. 2000; 27:329-337.

In summary, undue experimentation is not required to make and use antibody fragments or synthetic peptides which retain specific antigen-binding activity because: (1) the antigen-binding portion of an antibody has a stable conformation that is not likely to be affected by limited proteolysis or other methods of producing fragments; (2) predictable and well-known methods are available to prepare a variety of antibody fragments such as Fab, F(ab')$_2$, or Fv, with retention of the antigen-binding conformation; (3) there is no reason to expect disruptive interactions between the ligand and the oligopeptide; (4) any ligands in which unpredicted structural alterations arise can be screened out using a routine antigen binding assay; and (5) critical amino acid residues in a binding region of the ligand.can be protected during coupling to the oligopeptide.

For the reasons discussed above, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 1-4, 8, 11-21, and 44 Under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 8, 11-21, and 44 stand rejected for alleged lack of written description of ligands consisting of fragments of antibodies or synthetic polypeptides. The office action asserts that the specification merely mentions antibody fragments and synthetic polypeptides, which is insufficient to support the generic claims under the Interim Written Description Guidelines. The rejection is respectfully traversed.

The Interim Written Description Guidelines state that the written description requirement is satisfied for a claimed genus through the description of sufficient relevant identifying characteristics to show that the applicant was in possession of the genus. Any appropriate combination of characteristics can be used. Structure is not the only descriptor available to the applicant. The species within the genus can also be described in terms of their function and in terms of structure-function relationships.

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by relevant identifying characteristics, *i.e.*, structure or other physical and/or chemical characteristics, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus.

PTO Interim Guidelines on Written Description, 63 Fed. Reg. 32639 (1998); Patent, Trademark & Copyright Journal Vol. 56, 219, 222 (1998). Thus, the written description requirement is met if sufficient identifying characteristics are presented to demonstrate that the applicant was in possession of the claimed genus. Here, Applicant has provided description of the ligand's structure, function, and structure-function relationships, as described below.

(1) Structure. The genus in question includes ligands that specifically bind a cell surface antigen. The ligand of the subject claims comprises an antibody, a fragment of an antibody, or a synthetic polypeptide. The relevant structure of the ligand is the antigen-binding moiety of an antibody or a synthetic polypeptide. No other specific structural features are relevant to the invention. Therefore, description of further structural detail is not relevant.

The claimed genus covers antibodies, fragments of antibodies, and synthetic peptides which specifically bind to a cell surface antigen. The structure of one specific antibody, Mab L8A4, is specified *inter alia* in the specification at page 12, line 28 through page 13, line 12; in Example 6; in Figures 2-6; and in several publications referenced in the specification (*e.g.*, Reist et al. (1995) and Reist et al. (1996)). Two further antibodies, Y10 and H10, are specified at page 6, line 6, and by reference to Reist et al. (1995). Additional structural features are presented in the last paragraph on page 5, which describes the antibody as belonging to any of five structural classes (IgG, IgA, IgD, IgE, or IgM), as being either polyclonal or monoclonal, and as being harvested from certain species of mammal. Still other structural aspects are described for certain embodiments in which the ligand is an interspecies antibody. Such embodiments might combine, for example, a constant domain of a human immunoglobulin with variable regions from another species containing a specific antigen binding site. *See, e.g.*, specification at page 13, lines 14-25.

Use of the term "antibody fragment" in connection with specific antigen binding automatically conjures up in the mind of the ordinary skilled artisan a whole palette of standard fragments, such as Fab, $F(ab')_2$, and single chain Fv fragments. It is unnecessary to describe the detailed structural characteristics of these and similar fragments because they are well known in the art. Nonetheless, the specification at page 13, lines 6-12 describes the preparation of single

chain Fv fragments of Mab L8A4 with details on the linking peptide and the antigen binding affinity.

> Single chain F_v (scF_v) constructs based on the L8A4 variable region can also be used instead of the whole L8A4 antibody. An scF_v monomer was labeled with SIPC and found to have a K_A of 1.5×10^8 M^{-1} and an immunoreactive fraction of 65-80%. Multivalent constructs have been created by varying the length of the linker between the V_L and V_H domains; with a 5 amino acid linker, a dimer has been generated with a K_A of 5.5×10^9 M^{-1} measured by surface plasmon resonance.

The specification also references U.S. Patent 5,260,203, which describes the structure of other single chain Fv fragments in great detail.

(2) Function. The ligand is described as specifically binding to a cell surface antigen. This can be found in the original claim as well as in the specification *inter alia* at page 5, lines 12-13 ("A ligand, as the term is applied to this invention, is any molecule which specifically binds to a cell surface antigen.") and page 5, lines 17-18 ("A ligand is considered to bind specifically when it binds with an affinity constant of 10^6 M^{-1} or more, preferably 10^8 M^{-1} or more."). Another function of the ligand is to be covalently linked to an oligopeptide. This is described in the specification *inter alia* at page 9, lines 11-13: "a composition of the invention comprises a ligand that is covalently bound to an oligopeptide". Yet another function of the ligand is to be internalized by a cell. This is described *inter alia* at page 5, lines 14-15: "Ligands can be internalized by the cell over seconds, minutes, hours, or days".

(3) Relation of Structure to Function.

The specification teaches that the antigen binding site should be preserved in any antibody fragment used in the ligand, and that portions of both the light and heavy immunoglobulin chains are retained in the fragment.

> If a fragment of an antibody is used, the fragment should be capable of specific binding to a cell surface antigen. The fragment can comprise, for example, at least a portion of an immunoglobulin light chain variable region and at least a portion of an immunoglobulin heavy chain variable region.

Specification at page 6, lines 13-17. Further, the specification teaches that synthetic polypeptides can be used directly if they bind a cell surface antigen, and that synthetic polypeptides also can be employed to join light and heavy chain variable regions in a single chain Fv fragment.

> A ligand can also be a synthetic polypeptide which specifically binds to a cell surface antigen. For example, the ligand can be a synthetic polypeptide comprising at least a portion of an immunoglobulin light chain variable region and at least a portion of an immunoglobulin heavy chain variable region, as described in U.S. Patent 5,260,203 or as otherwise known in the art.

Specification at page 6, lines 17-21.

The Interim Guidelines on Written Description state that where an art is well developed, and identifying characteristics are well known for a substantial portion of a genus, the applicant's disclosure must be combined with what is known in the art in order to assess whether the description requirement has been met. An example is provided of a claim to a genus of monoclonal antibodies with defined antigen-binding characteristics. The Guidelines state:

> Although the specification does not disclose the complete structure of a representative number of species to support the claimed genus of antibodies, it does disclose multiple monoclonal antibodies which have the isotype claimed as well as the binding specificity and binding affinity characteristics recited in the claims. In this well-developed art, additional identifying characteristics for a substantial portion of the genus are well-known (*e.g.*, number of chains, disulfide bonds, constant and variable regions, etc.). Thus, applicant's disclosure combined with what was known in the art are sufficient to describe the claimed genus of monoclonal antibodies in such full, clear, concise and exact terms to show applicant was in possession of the claimed antibodies.

Patent, Trademark & Copyright Journal Vol. 56, 219, 223. This example closely resembles the situation for the ligand of the subject claims. The production and use of antibody fragments and synthetic polypeptides which bind cell surface antigens is routine technology, and when the published teachings for that technology are combined with the disclosure of the subject application, it is clear that Applicant was in possession of the entire genus.

For the reasons discussed above, the withdrawal of this rejection is respectfully requested.

Allowance of all pending claims is respectfully requested.

Respectfully submitted,

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